PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.2917

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

Advanced Mutant Line Developed from Fatemadhan Shows Salinity Tolerance at both Seedling and Reproductive Stages

Tasnia Khatun[1], Sheikh Mahfuja Khatun[1], Jannatul Naim[1], Sopnil Ahmed Jahin[1], Mumtarin Haque Mim[1], Mohammad Rashed Hossain[1], Mohamed A. El-Esawi[2, 3] & Mohammad Anwar Hossain[1*]

[1]Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
[2]Botany Department, Faculty of Science, Tanta University, Tanta 31527, Egypt
[3]Department of Biological Sciences, Faculty of Science, New Mansoura University, New Mansoura City, Egypt

***Email:** anwargpb@bau.edu.bd

ARTICLE HISTORY
Received: 01 September 2023
Accepted: 13 October 2023
Available online
Version 1.0 : 15 December 2023

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS,UGC Care etc.
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE
Khatun T, Khatun S M, Naim J, Jahin S A, Mim M H, Hossain M R, El-Esawi M A & Hossain M A. Advanced Mutant Line Developed from Fatemadhan Shows Salinity Tolerance at both Seedling and Reproductive Stages. Plant Science Today (Early Access). https://doi.org/10.14719/pst.2917

Abstract

The generation of high-yielding rice mutants and their assessment under salt stress offers a great possibility to isolate salt tolerant line(s) with desired trait of interest. Two separate experiments were conducted at the seedling and reproductive stages of rice to assess the level of salinity tolerance of few advanced high-yielding rice mutants. In the first experiment, rice seedlings were grown under hydroponic conditions and 14-day-old seedlings were subjected to salt stress (EC=10 dS/m; 7 days). Salt stress caused significant reduction in root and shoot length and biomass and leaf chlorophyll content; however, a little reduction was found in the mutant Line-1. In contrast, a sharp increase in shoot Na^+/K^+ ratio was found in all the genotypes except, Binadhan-10, FL-478 and the mutant Line-1, which exhibited little increased ratio. The second experiment involved exposure of plant to salt stress (EC=10 dS/m) for three weeks at the late booting stage in a sizable plastic tub filled with field soil. Salt stress resulted in a significant decrease in yield and yield attributing traits in all the genotypes except Binadhan-10. Grain yield per panicle was found significantly positive correlation with panicle length, the number of filled grains per panicle, and 100-seed weight under both control and salt stress conditions. Based on the studied traits and stress tolerance indices, Binadhan-10 and mutant Line-1 categorized as salt tolerant and rest of the genotypes were categorized as susceptible, which is also evident from the biplot of principal component analysis. Considering the results from both of the experiments, mutant Line-1 was found tolerant genotype at both seedling and reproductive stage. However, further studies are required to determine the genetic issues controlling the salinity tolerance in mutant Line-1 and the high-yield potential of mutant Line-65 under control condition in a way to develop salt tolerant and high-yielding rice varieties, respectively.

Keywords

Rice mutants; phenotypic variability; salinity tolerance; correlation co-efficient; principal component analysis; stress tolerance indices

Introduction

More than half of the world's population consumes rice (*Oryza sativa* L.), the second-most significant grain crop in the world, primarily in East and Southeast Asia (1). By 2050, it is expected that there will be more than 9.6 billion people on Earth, requiring an estimated increase of food grain production by 70% (2) and rice production by 87%. Bangladesh produces 56.41 Mt of rice annually on 11.91 Mha of land, ranking fourth in the world (3). In Bangladesh, rice cultivation takes up around 70% of all farmed land. Different levels of soil salinity impact 53% of Bangladesh's coastal areas, especially during the dry season (November to May), which normally limits crop productivity throughout the year (4). These regions have exceptionally low agricultural land utilization, significantly lower than the national average for crop intensity. Moreover, further increase in soil salinity is projected to result in 15.6% decrease in rice yield by 2050 (5). Due to the fact that salt intrusion is a significant problem for rice cultivation in Bangladesh's coastal region, it is now crucial to investigate the potential uses of the salt -tolerant germplasm resources by avoiding fallow land during the rabi season through the development of suitable high-yield salt-tolerant rice varieties to meet the demand of the expanding population. Therefore, it is imperative to develop rice cultivars that can withstand extreme salt stress and continue to yield well in both saline and non-saline conditions.

Salt stress is a complicated process that impairs a plant's physiological and biochemical functions. The effect of salt stress is exerted in all stage of rice cultivation; starting from seed germination to flowering and fruiting; resulting in lower seed set and diminishing return of the crop products (6). Reactive oxygen species (ROS) generation increases as a result of osmotic and ionic stress brought on by salt stress that damage important molecule, namely DNA, proteins and cell membranes (7). Salt stress decreases nutrient uptake and enzyme activity, which has a considerable negative impact on growth and yield. Additionally, salt stress reduces the effectiveness of photosynthetic processes and increases the buildup of harmful ions (Na^+), which result in significant growth and yield losses (8). Na^+ and K^+ compete for absorption into the roots when exposed to salt stress. Na^+ excess in soils can result in K^+ deficiency by preventing K^+ uptake into plant cells and causing its efflux from cells. Plants exhibit numerous adaptive physiological and biochemical mechanisms in order to combat the adverse effects of salt stress (9). By using salt exclusion, salt dilution, leaf-to-leaf compartmentalization, salt re-absorption, and Na^+ partitioning, salt-tolerant plants often maintain a low Na^+/K^+ ratio under saline conditions (10). Thus, the Na^+/K^+ ratio is an important determinant of salt tolerance in rice. One typical tactic of salinity tolerance is the maintenance of turgor pressure by the synthesis, transport, and aggregation of low-molecular weight osmolytes (11).

Rice is a salt-sensitive crop, but the level of sensitivity varies with various growth and development phases. It is particularly highly sensitive at the early seedling stage and reproductive stage (12). Further evidences suggest that these two sensitive stages in rice are independent from each other and are controlled by different gene sets is provided by the lack of a correlation between tolerance at the seedling and reproductive phases (12). Therefore, it is not a good idea to develop a variety of rice based solely on salinity tolerance at the seedling stage or at the reproductive stage. Due to our incomplete understanding of the molecular and genetic mechanisms underlying salt stress resistance and the lack of effective phenotyping and genotyping procedures, conventional breeding to boost crop yields in saline areas is frequently delayed. In order to develop a robust salt-tolerant genotype in rice, the assessment and isolation of genotypes at various phases of growth under controlled condition is essential to minimize the environmental effects.

Fatemadhan, a high yielding novel rice genotype has been selected from a hybrid rice variety. Compared to other traditional varieties, the selected genotype has better phenotypic traits such as significantly higher number of spikelets per panicle, very long panicle, strong and stout stem and larger (in terms of length and breadth) flag leaf. This prospective genotype does, however, also includes a number of undesirable traits, like tall plant, irregular flowering and maturation, a long awn, an increase in chaffy grains, etc. However, this genotype could be a fantastic starting point for a rice breeding program that aims to create high yielding rice types. Induced mutation breeding can be very effective in this case for altering those unfavourable traits and developing mutant lines with higher yield potential and tolerance to biotic and abiotic stresses. Induced mutations are important sources of genetic variability that can be applied artificially (13). In the case of rice, gamma rays can produce mutants that can tolerate abiotic stresses like salt and drought (13). In our lab, we have produced a large number of mutant lines from Fatemadhan through physical and chemical mutagenesis (14). Importantly, few selected lines were found to have higher yield potential compared to the commercially cultivated varieties. In addition, it would be worthwhile to investigate the salt tolerance attributes of those high yielding advanced mutant (M_4) lines. This research is thus carried out to assess the salinity tolerance of few advanced rice mutants based on phenotyping of key morphological and biochemical traits at both seedling- and -reproductive stages to identify potential mutants for developing high yielding and salinity tolerant varieties in future.

Materials and Methods

Screening of Rice Genotypes for Salt Stress Tolerance at the Seedling Stage

Experimental Materials

A total of six genotypes including three advanced mutant lines of Fatemadhan (Line-1, Line-18, and Line-65), one seedling stage salt tolerant advanced breeding line (FL-478), one salt tolerant variety (Binadhan-10) and one salt sensitive variety (BRRI dhan28) were used as plant materials. The Department of Genetics and Plant Breeding, Bangladesh Agricultural University (BAU), Bangladesh Institute of Nuclear Agriculture (BINA), and Bangladesh Rice Research Institute (BRRI) provided the seeds for these genotypes.

Design and Treatments of the Experiment

The experiment was carried out in the glasshouse facility of BINA, Mymensingh-2202, Bangladesh, using a completely randomized design (CRD) with hydroponic conditions, three independent replications, and two treatments: control and salt stress (EC=10 dS/m).

Seedling Growth & Salt Stress Treatment in Hydroponic System

Seeds were surface sterilized with 70% ethanol, placed on wet filter paper in petri dishes for 24 h, and then incubated for 3-4 days in the dark to promote sprouting. Sprouted seeds were then sown in a line into the holes of a styrofoam sheet floating in a rectangular plastic tray (having dimensions of 32.50 cm × 28.50 cm × 13.00 cm length, breadth and width) containing distilled water (Fig. 1). After 4 days of seedlings growth, the water of the trays was replaced with Peters® Professional (Geldermalsen, Netherlands) solution. The solution's pH was kept between 5.0 and 5.1. An air pump was used to stir the fluid, ensuring a constant flow of nutrients to the plants. After every seven days, the nutrition solution was replaced.

After two weeks of seedling growth in hydroponic solution, they were exposed to salinity stress (10 dS/m) by adding sea salt solution in the nutrient solution and continued for 7 days. By regulating the Electrical Conductivity (EC) of the nutrient solution using an EC meter (Hanna HI 4321, Weilheim, Germany), the salinity level was maintained. The control plants were raised in a salt-free nutrient solution.

Data on Morphological and Biochemical Traits

Ten seedlings per genotype were observed after seven days of salt stress treatment for shoot length (SL), root length (RL), shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), and relative chlorophyll content (SPAD units).

Determination of Na^+: K^+ ratio

Following a week-long treatment with salt stress, rice plants were divided into roots and shoots, which were then dried for three days at 60°C to maintain weight. In a micro-Kjeldahl digesting system, oven-dried materials were ground to a fine powder using a mortar and pestle following the method as described by Thomas *et al.* (15). A flame photometer (Model PERKIN-ELMER, 2380) was used to measure the Na^+ and K^+ in the digested samples. Na^+ and K^+ concentrations in plant tissues were used to calculate the Na^+: K^+ ratio.

Screening of Rice Genotypes for Salt Stress Tolerance at the Reproductive Stage

Experimental Materials

All the genotypes of experiment I except FL-478 were used as plant materials.

Design and Treatments of the Experiment

At the net house facility of the Department of Genetics & Plant Breeding, BAU, Mymensingh-2202, Bangladesh, the experiment was carried out using a randomized complete block design (RCBD) with three replications and two treatments, control and salt stress (EC=10 dS/m).

Plastic-tub Preparation, Seedling Transplanting & Imposition of Salt Stress

The experiment was conducted in a large size plastic-tub (135 cm × 90 cm × 24 cm) filled with field soil. The soil of the tub was prepared by puddling the soil and mixing the recommended doses of cow dung, fertilizers (Urea, TSP, and MP). Thirty days old seedlings were transplanted in the plastic tub. Two seedlings were transplanted per hill keeping plant to plant distance of 20 cm and row to row distance of 25 cm. At the end of rice growth stage 4 (young panicle about to emerge from flag leaf), leaf clipping of each genotype was performed while leaving the flag leaf. Saline water was then irrigated for three weeks at a level of one centimetre above the soil surface. After three weeks, plants were irrigated with normal irrigation water. Data were recorded after harvesting.

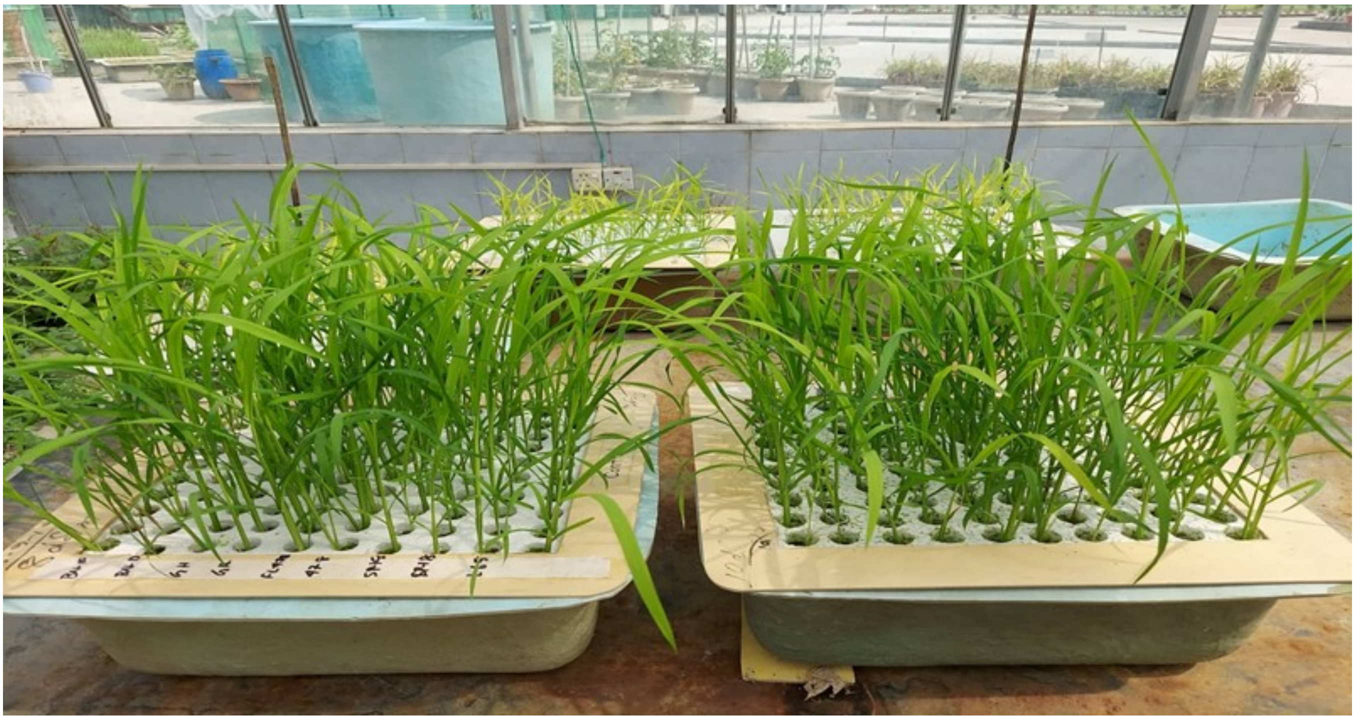

Fig 1: Partial view of the hydroponic screening of rice seedling for salt tolerance at the seedling stage.

Data Collection on Yield Related Traits

Ten randomly chosen plants from each genotype were used to gather information on yield and traits that contribute to yield, including days to maturity (DM), plant height (PH), panicle length (PL), number of filled grains per panicle (NFGP), number of unfilled grains per panicle (NUGP), spikelet fertility percentage (SF%), 100-seed weight (100-SW), and grain yield per panicle (GYP).

Stress Tolerance Indices

Stress tolerance indices such as MP (Mean Productivity), GMP (Geometric Mean Productivity), SSI (Stress Susceptibility Index), TOL (Tolerance Index), STI (Stress Tolerance index) and YSI (Yield Stability Index) were estimated in grain yield per panicle using following equations: $GMP = \sqrt{(Yp \times Ys)}$ (16); $MP = (Yp + Ys)/2$ (17); $SSI = (1 - (Ys/Yp))/(1 - (\bar{Y}s/\bar{Y}p))$ (18); $STI = (Yp \times Ys)/(\bar{Y}p)^2$ (16); $TOL = Yp - Ys$ (17); $YSI = Ys/Yp$ (19).

Where, Ys and Yp indicate yield per panicle of a given genotype under stress and normal condition, respectively.

Statistical Analysis of the Data

All statistical analyses were done by MINITAB 19 (Minitab Inc., State College, Pennsylvania) following CRD design for the seedling stage experiment and RCBD design for the reproductive stage experiment. The Tukey's multiple comparison test was used to determine whether there was a significant difference in treatment means at the $P \leq 0.05$ level. *, **, and *** indicates significant at 5, 1 and 0.1% levels of probability.

Results

Effect of Salt Stress on Rice Genotypes at the Seedling Stage

The result of analysis of variance (ANOVA) showed highly significant variation among genotypes (G), treatments (T) and G × T for all the traits studied (Supplementary Table 1).

Shoot Length (SL)

Under control condition, maximum SL was observed in FL-478 (52.47 cm) whereas minimum was observed in Line-18 (40.73 cm) (Table 1). Salt stress resulted in a significant decrease in SL in all the genotypes studied. The highest reduction was observed in Line-65 (30.12%) followed by BRRI dhan28 (25.58%), FL-478 (25.52%), Line-1 (23.32%), Binadhan-10 (20.76%), and Line-18 (20.62%) when compared with control (Table 1).

Root Length (RL)

Significant variation was observed among the genotypes for RL at both control and stressed condition. The highest RL was found in Binadhan-10 (14.97 cm) whereas the lowest was found in Line-18 (10.90 cm) under control conditions (Table 1). Salt stress resulted in significant reduction in RL among all of the genotypes; the highest reduction was found in Line-18 (21.74%) followed by Line-65, BRRI dhan28, Binadhan-10, Line-1, and FL-478 (20.11%, 18.61%, 15.50%, 13.08% and 8.88%, respectively) as compared to control.

Shoot Fresh Weight (SFW)

Both under control and salt stress conditions, a striking variation in SFW was seen. Under control conditions, FL-478 (1.74 g) had the highest SFW while BRRI dhan28 (0.69 g) had the lowest SFW (Table 1). Most genotypes experienced a striking reduction in SFW as a result of salt stress. When compared to control, the BRRI dhan28 showed the greatest reduction (57.97%), followed by Line-18, Line-65, FL-478, Binadhan-10, and Line-1 (53.85%, 49.59%, 49.43%, 41.18, and 27.68%, respectively).

Root Fresh Weight (RFW)

FL-478 had the highest RFW under control conditions (0.48 g), while BRRI dhan28 had the lowest (0.13 g). In response to salt stress, there was a significant heterogeneity among genotypes for RFW (Table 1). The maximum reduction in RFW was seen in Line-1 (25.81%), followed by BRRI dhan28, Line-18, Binadhan-10, Line-65, and FL-478 (25.38%, 19.05%, 12.90%, 12.5%, and 6.25%, respectively), when compared to control.

Shoot Dry Weight (SDW)

Under both control and salt stress conditions, there was a noticeable variation among SDW among the genotypes under study. In the control condition, FL-478 had the highest SDW (267.84 mg), while BRRI dhan28 had the lowest SDW (119.85 mg) (Table 1). All genotypes showed a significant decrease in SDW in response to salt stress; the genotype with the highest reduction, BRRI dhan28 (40.38%), was followed by Line-65, FL-478, Line-18, Binadhan-10, and Line-1 (39.72%, 37.37%, 30.22%, 20.77%, and 5.83%, respectively), all of which were significantly lower than the control.

Root Dry Weight (RDW)

Similar to SDW, significant variation was also observed among the studied genotypes in case of RDW. The highest RDW was noted in FL-478 (43.51 mg) and the lowest was recorded in BRRI dhan28 (19.56 mg) under control conditions (Table 1). Imposition of salt stress led to a significant decrease in RDW, maximum decrease in RDW was recorded in BRRI dhan28 (43.15%) followed by Line-18, Line-1, FL-478, Binadhan-10 and Line-65 (27.22%, 24.20%, 16.55%, 16.14% and 13.03%, respectively) as compared to control.

Relative Chlorophyll Content (SPAD value)

The SPAD value showed significant variation under both control and salt stress conditions. Under control condition, the highest SPAD value was found in FL-478 (38.70) while lowest in Line-18 (33.07). Salt stress resulted in a considerable damage to chlorophyll content as compared to control (Table 1). The highest damage in chlorophyll content was noted in BRRI dhan28 (31.98%) followed by Line-18, Line-65, Line-1, Binadhan-10 and FL-478 (26.22%, 23.17%, 15.53%, 15.44% and 3.23%, respectively).

Shoot Na^+: K^+(S Na^+: K^+)

Under both control and salt stress conditions, there was a considerable variation in S Na^+: K^+ among the genotypes. Line-1 had the lowest value (0.11) and Binadhan-10 had the greatest value (0.32). The Na^+: K^+ in the shoot tissue was significantly induced by the application of salt stress (Table 1). The maximum induction was seen in BRRI dhan28 (992.86%), followed by Line-18 (481.25%), Line-65 (407.69%), Line-1 (218.18%), FL-478 (158.33%) and Binadhan-10 (18.75%), when compared to control.

Root Na^+: K^+(R Na^+: K^+)

Regardless of the treatments, R Na^+: K^+ genotypes significantly varied during both control and salt stress conditions. Under the control treatment, the maximum value was seen in BRRI dhan28 (0.26) and the minimum value in Line-1 (0.15) (Table 1). Salt stress imposition resulted in significant induction of R Na^+: K^+ as compared to control. As compared to control, the highest induction was found in Line-18 (20.52-fold) followed by Line-1, FL-478, Binadhan-10, BRRI dhan28 and Line-65 (20.46, 19.45, 12.12, 11.11 and 1.6-fold, respectively).

Effect of Salt Stress Yield and Yield-Attributing Traits at the Reproductive Stage

Analysis of variance (ANOVA) results revealed highly significant variation for all examined traits when considering genotype (G), treatment (T) and G × T (Supplementary Table 2).

Days to Maturity (DM)

For the trait DM, there was significant heterogeneity among the genotypes (Table 2). Under control conditions, Line-18 took the most days (144 days) to reach maturity while BRRI dhan28 needed a shorter period of time (131 days). All of the genotypes showed a considerable reduction in DM as a result of salt stress. The Line-1 (6.62%) and Line-65 (6.06%), BRRI dhan28 (5.34%), Line-18 (4.86%) and Binadhan-10 (1.52%) lines experienced the largest declines.

Plant Height (PH)

The genotypes at both control and stressed settings showed substantial variation in PH. Under control treatment, Line-18 (85.62 cm) showed the highest PH, and Line-1 (68.77 cm) showed the lowest PH (Table 2). When salt stress was applied, the PH significantly decreased as compared to the control. The reduction that was determined to be the greatest was in the BRRI dhan28 (18.12%), followed by the Binadhan-10, Line-1, Line-18, and Line-65 (17.96%, 17.32%, 10.84% and 9.73%, respectively).

Panicle Length (PL)

Significant variation was observed among the genotypes for PL both under control and salt stress conditions (Table 2). The highest PL under control condition was found in Line-65 (28.52 cm) whereas the lowest was found in BRRI dhan28 (23.15 cm). Exposure of rice plants to salt stress resulted in a considerable reduction in PL, however, the highest reduction was observed in, Line-18 (11%) followed by BRRI dhan28, Line-1, Binadhan-10 and Line-65 (10.45%, 10.35%, 9.33% and 6.21%, respectively) as compared to control.

No. of Filled Grains per Panicle (NFGP)

Under both control and stress conditions, the genotypes varied remarkably in terms of NFGP. Under control condition, the highest NFGP was found in Line-65 (258) and the lowest in Binadhan-10 (96) (Table 2). Salt stress led to a considerable decrease in NFGP in all the studied genotypes. As compared to control, the highest reduction was noted in Line-65 (74.42%) followed by Line-18, BRRI dhan28, Line-1, and Binadhan-10 (73.88%, 71.21%, 64.23% and 30.21%, respectively).

No. of Unfilled Grains per Panicle (NUGP)

A marked variation was observed among the genotypes for the traits NUGP under both treatments. The highest NUGP was noted in Line-18 and the lowest was recorded in BRRI dhan28 (Table 2). Imposition salt stress resulted in a significant induction in NUGP as compared to control. The highest induction was found in BRRI dhan28 (745.45%) followed by Line-1, Line-65 Line-18 and Binadhan-10 (125.23%, 124.83%, 66% and 42.86%, respectively).

Spikelet Fertility Percentage (SF%)

The genotypes showed significant variation in respect to SF% with a range from 47.18 to 92.31. BRRI dhan28 showed the highest value (92.31) for SF% whereas Line-18 showed the lowest value (47.18) for SF% (Table 2). Salt stress led to a significant decrease in SF% among all the

Table 1: Performances of six rice genotypes for different traits related to yield under control and salt stress (10 dS/m) conditions at the seedling stage. Data represented in the table are the treatment means of three replicates (10 plants per replication).

Genotype	Treat-ment	SL (cm)	RL (cm)	SFW (g)	RFW (g)	SDW (mg)	RDW (mg)	SPAD	S Na^+:K^+	R Na^+:K^+
BRRI dhan28	Control	43.67CD	12.20A-D	0.69EF	0.13DE	119.85F	19.56E	36.68A-C	0.14D	0.26EF
	Salt	32.50G	9.93CD	0.29H	0.097E	71.46G	11.12F	24.95F	1.53A	2.89C
Binadhan-10	Control	45.37C	14.97A	1.19B	0.31BC	173.48BC	28.43C	38.35A	0.32CD	0.16FG
	Salt	35.95FG	12.65A-C	0.70EF	0.27CD	137.45E	23.84DE	32.43CD	0.38CD	1.94D
Line-1	Control	50.60AB	14.53AB	1.12BC	0.31BC	165.15CD	26.98CD	37.28AB	0.11D	0.15G
	Salt	38.80EF	12.63A-C	0.81DE	0.23C-E	155.53D	20.45E	31.49DE	0.35CD	3.07B
Line-18	Control	40.73DE	10.90B-D	1.04C	0.21C-E	160.14D	20.54E	33.07B-D	0.16D	0.21FG
	Salt	32.33G	8.53D	0.48G	0.17C-E	111.74F	14.95F	24.40F	0.93B	4.31A
Line-65	Control	47.37BC	13.23A-C	1.23B	0.24C-E	181.11B	22.49E	35.69A-D	0.13D	0.23FG
	Salt	33.10G	10.57CD	0.62F	0.21C-E	109.17F	19.56E	27.42EF	0.66BC	0.37E
FL-478	Control	52.47A	11.60AB-D	1.74A	0.48A	267.84A	43.51A	38.70A	0.12D	0.22FG
	Salt	39.08EF	10.57CD	0.88D	0.45AB	167.75CD	36.31B	37.45AB	0.31CD	4.28A

Note: Different letters in the same column are significant at 5% level of probability following Tukey's method. (Here, SL= shoot length; RL= root length; SFW= shoot fresh weight; RFW= root fresh weight; SDW= shoot dry weight; RDW= root dry weight; SPAD= soil plant analysis development; S Na^+:K^+= shoot Na^+: K^+ ratio; and R Na^+: K^+= root Na^+: K^+ ratio genotypes studied when compared with the control. The highest reduction was observed in Line-65 (74.03%) followed by Line-18, BRRI dhan28, Line-1 and Binadhan-10 (73.89%, 68.57%, 61.62% and 11.76%, respectively).

100-Seed Weight (100-SW)

A remarkable variation was observed in 100-SW under control and salt stress conditions. On average, 100-SW was the highest in Line-65 (2.84 g) and the lowest in BRRI dhan28 (2.17 g) under control condition. Salt stress causes considerable reduction in 100-SW (Table 2). Maximum reduction was found in BRRI dhan28 (32.26%) followed by Line-18, Binadhan-10, Line-65, and Line-1 (23.29%, 20.31%, 19.37% and 7.27%, respectively) relative to control.

Grain Yield per Panicle (GYP)

Significant variation was observed among the studied genotypes for grain yield per panicle both under control and salt stress conditions (Table 2; Fig. 2). The highest GYP was found in Line-65 (6.57 g) whereas the lowest was found in Binadhan-10 (2.46 g) under control condition (Table 2). Salt stress resulted in significant reduction in GYP among all of the genotypes. Compared to control, the highest reduction was found in Line-65 (80.82%) followed by BRRI dhan28, Line-18, Line-1 and Binadhan-10 (79.55%, 78.32%, 55.45% and 39.84%, respectively).

Phenotypic Correlation Co-Efficient among Eight Characters of Rice Genotypes under Control and Stress Conditions at the Reproductive Stage

Correlation analysis was carried out to estimate the correlations between the eight morphological traits of rice seedlings under salt stress (Table 3). Plant height showed a significant positive correlation with DM (0.528*) under salt stress condition. NFGP showed a significant positive correlation with PL (0.710*, 0.650*) and a significant negative correlation with PH (-0.515*, -0.597*) under both control and stress conditions (Table 3). In case of NUGP, it showed a significant positive correlation with DM (0.580*) and NFGP (0.630*) whereas it showed a significant positive correlation with PL (0.513*) under salt stress condition. SF (%) showed a significant negative correlation with DM (-0.80**) under control conditions whereas it showed a significant negative correlation with NUFP (-0.940***, -0.831***) both under control and salt stress conditions (Table 3). The relationship of 100-seed weight with PH and PL was found positively significant (P≤.05) under control whereas 100-SW had positive correlation with PL and NFGP at 0.1% and 5% level of probability respectively under saline condition. Finally, GYP showed a significant positive correlation with PL (0.72**, 0.678**), NFGP (0.82**, 0.953***), and 100-SW (0.57*, 0.643**) both under control and salt stress conditions whereas it showed significant positive correlation with NUFP (0.70**) under control conditions (Table 3).

Table 2: Performances of five rice genotypes for different morphological traits related to yield grown under control and salt stress (10 dS/m) conditions at the reproductive stage. Data represented in the table are the treatment means of three replicates (10 plants per replication).

Genotype	Treatment	DM	PH (cm)	PL (cm)	NFGP	NUGP	SF %	100-SW (g)	GYP(g)
BRRI dhan28	Control	131.00CD	77.16BC	23.15DE	132.00C	11.00F	92.31A	2.17C-E	2.69CD
	Salt	124.00F	63.18F	20.73F	38.00F	93.00E	29.01G	1.47F	0.55G
Binadhan-10	Control	132.00C	80.67AB	26.37A-C	96.00D	14.00F	87.27B	2.61AB	2.46C-E
	Salt	130.00D	66.18EF	23.91DE	67.00E	20.00F	77.01C	2.08DE	1.48E-G
Line-1	Control	136.00B	68.77DE	27.33A	246.00B	107.00D	69.69D	2.20C-E	4.04B
	Salt	127.00E	56.86G	24.50CD	88.00D	241.00B	26.75G	2.04DE	1.80D-F
Line-18	Control	144.00A	85.62A	24.89B-D	134.00C	150.00C	47.18F	2.49BC	3.46BC
	Salt	137.00B	76.34BC	22.16EF	35.00F	249.00B	12.32H	1.91E	0.75E-G
Line-65	Control	132.00CD	80.03B	28.52A	258.00A	149.00C	63.39E	2.84A	6.57A
	Salt	124.00F	72.24CD	26.75AB	66.00E	335.00A	16.46H	2.29B-D	1.26FG

Note: Different letters in the same column are significant at 5% level of probability following Tukey's method. (Here, DM= days to maturity; PH= plant height; PL= panicle length; NFGP= number of filled grains per panicle; NUGP= number of unfilled grains per panicle; SF%= spikelet fertility percentage; 100-SW= 100-seed weight; and GYP= grain yield per panicle. Different letters are significant at 5% level of probability following Tukey's method)

Table 3. Correlation co-efficient among eight characters of rice genotypes under control and salt stress condition at the reproductive stage.

Traits		DM	PH (cm)	PL (cm)	NFGP	NUFP	SF (%)	100-SW (g)
PH (cm)	Control	0.354						
	Salt	0.528*						
PL (cm)	Control	-0.182	-0.212					
	Salt	-0.315	0.071					
NFGP	Control	-0.132	-0.515*	0.710**				
	Salt	-0.405	-0.597*	0.650**				
NUGP	Control	0.580*	0.153	0.511	0.630*			
	Salt	-0.025	0.326	0.513*	0.148			
SF (%)	Control	-0.80***	-0.331	-0.315	-0.359	-0.940***		
	Salt	-0.040	-0.288	-0.013	0.280	-0.831***		
100-SW (g)	Control	-0.077	0.54*	0.61*	0.141	0.394	-0.335	
	Salt	0.004	0.198	0.791***	0.584*	0.439	0.075	
GYP (g)	Control	-0.104	-0.026	0.72**	0.82***	0.70**	-0.467	0.57*
	Salt	-0.205	-0.471	0.678**	0.953***	0.101	0.362	0.643**

Note: *, **, and *** indicates significant at 5, 1 and 0.1% levels of probability. (Here, DM=days to maturity; PH=plant height; PL= panicle length; NFGP=number of filled grains per panicle; NUGP=number of unfilled grains per panicle; SF=spikelet fertility; 100-SW=100-seed weight; and GYP=grain yield per panicle)

Principal Component Analysis (PCA)

Principal component analysis of all the morphological and biochemical parameters under unstressed (control) and salt stressed conditions in the five rice genotypes extracted three principal components (PC) with Eigen values greater than unity (Table 4). These three PCs explained 84.8% of the total variation in the datasets. PC1 accounted for 63.0% of the total variation which can be attributed to the higher contribution of SFW (0.294), SPAD value (0.285), SL (0.281), SDW (0.277), RDW (0.271), 100-SW (0.262), RL (0.259), YPP (0.254), PL (0.251) and NFGP (0.25) towards positive direction and S Na^+: K^+ ratio (-0.27), R Na^+: K^+ (-0.239) and NFGP (-0.113) towards negative direction (Table 4; Fig. 2). The second component, which explained 11.9% (Fig. 2) of the total variation, was mostly contributed by the higher positive loadings of SF% (0.428) and RL (0.296) and higher negative loadings of NUFG (-0.557), PL (-0.291), 100-SW (-0.259), DM (-0.254) and PL (-0.245).

Table 4. Component loadings of seventeen morphological and biochemical traits in control and salt stressed plants of five rice genotypes as determined by the principle component analysis (PCA).

Variable	PC1	PC2	PC3
Shoot length (cm)	0.281	0.088	-0.047
Root length (cm)	0.259	0.296	0.229
SFW (g)	0.294	-0.113	0.044
RFW (g)	0.230	-0.001	0.344
SDW (mg)	0.277	-0.117	0.109
RDW (mg)	0.271	0.119	0.197
SPAD Value	0.285	0.218	-0.060
N^+/K^+ ratio Shoot	-0.270	0.046	0.011
N^+/K^+ ratio Root	-0.239	0.002	0.100
Days to Maturity	0.120	-0.254	-0.477
Plant height (cm)	0.140	-0.291	-0.526
Panicle length (cm)	0.251	-0.245	0.290
No. of filled grains	0.250	-0.107	-0.015
No. of unfilled grains	-0.113	-0.557	0.337
Spikelet fertility (%)	0.219	0.428	-0.226
100-seed weight (g)	0.262	-0.259	-0.027
Yield/panicle (g)	0.254	-0.183	-0.086
Eigenvalue	10.72	2.02	1.67
Proportion	63.0%	11.9%	9.8%
Cumulative	63.0%	74.9%	84.8%

Stress Tolerance Indices

From the grain yield per panicle, stress tolerance indices such as MP, GMP, SSI, TOL, STI, and YSI were calculated and shown in the Table 5. These selection criteria are suggested for choosing genotypes, taking into account plant yield, based on how well they performed in both normal and salt-stressed conditions. Line-65 recorded the highest MP (3.92) followed by Line-1 (2.92), Line-18 (2.11), Binadhan-10 (1.97) and BRRI dhan28 (1.62) (Table 5). The highest GMP was obtained for the genotype Line-65 (2.88) followed by Line-1 (2.70), Binadhan-10 (1.91), Line-18 (1.61) and BRRI dhan28 (1.22). The highest value for SSI was observed for the genotype Line-65 (1.16) followed by BRRI dhan28 (1.14), Line-18 (1.13), Line-1 (0.80) and Binadhan-10 (0.57). The greatest TOL was noted in the genotype Line-65 (5.31) followed by Line-18 (2.71), Line-1 (2.24), BRRI dhan28 (2.18) and Binadhan-10 (0.98) (Table 5). Maximum STI was recorded for the genotype Line-65 (0.56) followed by Line-1 (0.49), Binadhan-10 (0.25), Line-18 (0.18) and BRRI dhan28 (0.10). The highest YSI value was recorded for the genotype Binadhan-10 (0.60) followed by Line-1 (0.45), Line-18 (0.22), BRRI dhan28 (0.20) and Line-65 (0.19) (Table 5).

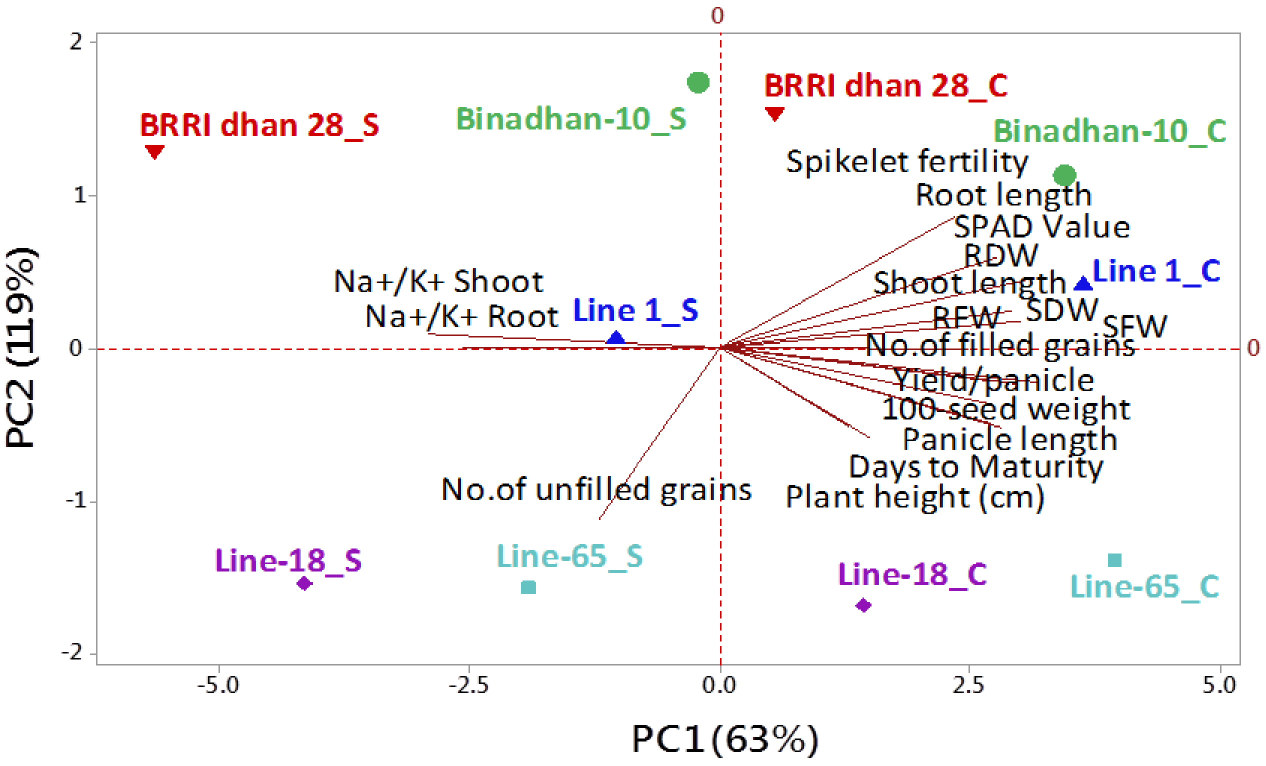

Fig 2: Biplot of seventeen morphological and biochemical traits in unstressed and salt stressed plants of five rice genotypes as determined by the principle component analysis (PCA). Different genotypes are indicated by different colors. The letters 'C' & 'S' followed by the genotype names represent control (unstressed) and salt-stressed condition, respectively.

Table 5: Stress Tolerance Indices in rice genotypes, estimated from grain yield/panicle obtained in control & salt stress condition.

Genotypes	MP	GMP	SSI	TOL	STI	YSI
BRRI dhan28	1.62	1.22	1.14	2.14	0.10	0.20
Binadhan-10	1.97	1.91	0.57	0.98	0.25	0.60
Line-1	2.92	2.70	0.80	2.24	0.49	0.45
Line-18	2.11	1.61	1.13	2.71	0.18	0.22
Line-65	3.92	2.88	1.16	5.31	0.56	0.19

Here, MP=Mean productivity; GMP=Geometric mean productivity; SSI=Stress Susceptibility Index; TOL= Tolerance Index; STI= Stress Tolerance Index; and YSI= Yield stability index

Discussion

Soil salinity can result in 20% to 100% reductions in rice yields (20). Salinity tolerance in rice varies depending on growth stages, duration and concentration of the salt stress. It's essential to comprehend the underlying mechanisms at various growth phases in order to breed genotypes that are salt-tolerant in all stages and can maintain higher yields. In the current study, three advanced mutant lines (Line-1, Line-18, Line-65) along with salt tolerant variety/line (Binadhan-10, FL-478) and salt susceptible check variety (BRRI dhan28) under saline condition (10 dS/m) were successfully screened based on seedling- and -reproductive stage-specific phenotyping protocol. Analysis of variances results showed highly significant differences for all the studied traits *viz*., SL, RL, SFW, RFW, SDW, RDW, SPAD value (chlorophyll content), S Na^+: K^+ ratio and R Na^+: K^+ratio in G, T and G × E interactions. These results indicated that all the genotypes had sufficient variability for these characters and there is scope of trait improvement through selection after assessing their subsequent generations.

Effect of Salt Stress on Rice Genotypes at the Seedling Stage

In this study, all genotypes of rice seedlings exposed to salinity showed a significant decrease in the growth and developmental indices such as SL and RL (Table 1). The highest reduction in SL and RL was observed in sensitive variety BRRI dhan28 and mutant Line-65 whereas the lowest reduction was observed in tolerant genotypes (FL-478 and Binadhan-10) and in the mutant Line-1. Numerous studies on rice were also reported genotypic variations in growth retardation in response to salt stress (21, 22). Plant growth is initially impeded by soil salinity by osmotic stress, but this is soon followed by ion toxicity (23). Osmotic stress initially results in a number of physiological changes, including membrane disruption, nutritional imbalance, diminished capacity to detoxify ROS, variations in antioxidant enzymes, and decreased photosynthetic activity (24). The excessive build-up of poisonous Na^+ in various plant tissues that interferes with water uptake and mineral homeostasis may be the source of damage to the growth of rice seedlings (25, 26). In addition, rice shoots showed higher sensitivity to salt stress than roots in all the salt stress environments studied, as salt stress can cause osmotic degradation in plant shoots and reduction in the number of viable leaves in rice seedlings (27).

A useful measure for evaluating the plants' resource -acquisition strategy is dry matter content. Based on the findings of the present research, the FW and DW of shoot, root and shoot biomass were significantly higher in all the genotypes under control conditions than those obtained under saline conditions (Table 1). The reduction in biomass under salinity stress is either caused by a lack of assimilates or by photosynthesis being inhibited or it might be brought on by the restriction of food hydrolysis and its transfer to growing shoots. Salinity stress resulted in a significant increase in Na^+ buildup, decreased photosynthetic pigments, and altered nutrient and water intake, which inhibited growth and the generation of biomass (28, 29). Similarly, tolerant genotypes (Binadhan-10, FL-478) and mutant Line-1 were shown to have the highest fresh and dry biomass whereas the sensitive variety (BRRI dhan28) and mutant lines (Line-18 and Line-65) have the lowest fresh and dry biomass under saline conditions. Similar to our results, the highest fresh and dry biomass in tolerant varieties and the lowest fresh and dry biomass in sensitive varieties under salt condition were observed by Rasel *et al.* (30). The phenological appearance of the seedlings also goes well with the results of the morphological traits where Line-1, Binadhan-10 and FL-478 showed better performance as compared to others (Fig. 3).

In relation to plant biomass and yield features, photosynthetic capacities in rice crops cultivated under salt stress are reported as sensitive metrics. By interfering with the function of photosynthetic pigments like "Chl a" and "Chl b," salt stress typically reduces the development and yield of crop plants and eventually, this results in plant death (31). Consequently, the amount of chlorophyll (Chl) in stressed plants can be used as a metric to examine how salinity affects various rice genotypes. In this study, salinity stress significantly reduced the Chl content (Table 1). The reduction of Chl content was highest in sensitive genotypes (BRRI dhan28, Line-18) and in mutant Line-65 compared to the tolerant genotype (FL-478, Binadhan-10) and mutant Line-1. The decreasing Chl content due to salinity was also observed by Moradi and Ismail (32) and Rahman *et al.* (33). Excessive Na^+ build-up denatures the enzyme required for Chl production, which lowers Chl synthesis. Due to salt toxicity, which mostly results in the burning of leaves or other succulent sections of salt-sensitive plant species and the destruction of other pigments as well, reduction of Chl content is a regular occurrence (34). Because of the limitation in diffusion brought on by stomatal closure, the decrease in Chl concentration may be one of the factors contributing to the reduction in photosynthesis by reducing the intercellular CO_2 availability (35). In the present study, the total Chl content was found to be significantly higher in the mutant Line-1 under salt stress condition (Table 1).

Based on SPAD values, Line-1 can be categorized as salt tolerant mutant as tolerant species can protect themselves from such deterioration of Chl under salinity stress. The phenological appearance of Line-1 seedlings grown under salt stress also reflected such phenomena (Fig. 3).

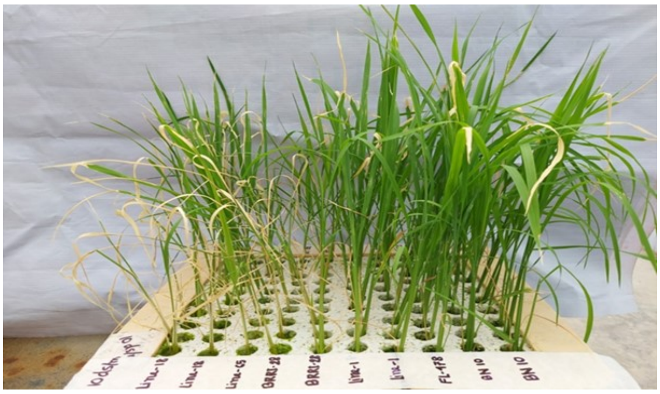

Fig 3. The phenological appearances of rice seedlings grown under 10dS/m salinity stress in hydroponic peter solution for one week.

One key factor in determining stress tolerance is the preservation of the Na^+/K^+ ratios. Except for some halophytic plants, sodium is a non-essential mineral element for plant growth and development. Ion toxicity, hyperosmotic, and oxidative stressors are all brought on by too much Na^+ in the soil in higher plants (36). In order to live in environments with higher NaCl concentrations, plants must either store or expel surplus Na^+ ions and maintain higher K^+ ions. Under extremely high salt conditions, ionic homeostasis or ion exclusion is a crucial defence mechanism to control the toxicity brought on by excess Na^+ (37). Under salt stress conditions, excessive Na^+ uptake through plant epidermal cells competes with the normal uptake of other nutritional ions, mainly K^+, and produces K^+ deficit, which results in greater Na^+/K^+ ratio and an imbalance of ionic homeostasis in rice. In salt-sensitive genotypes, accumulation of sodium to hazardous levels has been linked to a number of negative outcomes, including loss of membrane integrity and separation of plasma membrane from the cell wall (38), changed grana orientation, enlarged thylakoids, and distorted grana lamellae (38).

In this present research work, root Na^+/K^+ ratio was also significantly increased due to salt stress imposition. The highest induction was found in Line-18 followed by Line-1, FL-478, Binadhan-10, BRRI dhan28 and Line-65 (Table 1). From the comparison between shoot Na^+/K^+ ratio and root Na^+/K^+ ratio of the genotypes under salt stress, it was observed that BRRI dhan28 and Line-65 showed lower induction of root Na^+/K^+ ratio and higher induction of shoot Na^+/K^+ ratio whereas Line-18 showed higher induction for both shoot Na^+/K^+ ratio and root Na^+/K^+ ratio. This might be due to the highest Na^+ influx and translocation from root to shoot and the inability to exclude Na^+ indicating the sensitivity to salt stress of these genotypes. On the other hand, the tolerant genotypes (Binadhan-10 and FL-478) and mutant Line-1 have higher root Na^+/K^+ ratio yet these genotypes showed lower shoot Na^+/K^+ ratio. Better Na^+ efflux from roots to the rhizosphere via the well-known SOS1-dependent exclusion system; Na^+ sequestration in vacuoles is controlled by Na^+/K^+ antiporters; and Na^+ loading and unloading at the xylem are additional possible causes (39).

Effect of Salt Stress Yield and Yield-Attributing Traits at the Reproductive Stage

The effects of salt stress during the reproductive stage are more significant than those during the vegetative stage at the field level. Because phenotyping takes a lot of time and effort, it is one of the key reasons why there has been relatively less research on rice's ability to tolerate salinity during the reproductive stage. Yield is a complex phenotypic trait that largely depends on various yield and yield attributing traits. The results of the analysis of variance of the current study showed highly significant differences among the genotypes in all the studied traits due to salt stress which indicates that salt stress greatly affects all of the morphological traits which offers the possibility of improvement of yield through selection. Numerous studies on cereals and other crucial commodities for commerce found variations in yields in response to salinity stress (40, 41).

Increasing the YPP is the ultimate goal of rice breeder to increase rice production. Rice yield potential cannot be increased by enhancing a single yield feature. For instance, boosting grain production necessitates not only expanding the area of the sink by adding more panicles, but also adjusting other yield-contributing features like tiller number, PL, NFGP, SF%, grain weight per panicle, and 100-SW, among others. Rice yield and yield-related components are considerably decreased by salt stress throughout the reproductive stage (42). Rice yield can be reduced by 12% for every unit increase of EC above 3 dS/m (40). In our study, a significant decrease in yield attributing traits like DM, PH, PL, NFGP, SF%, 100-SW and YPP were observed in response to salt stress imposition where the NUFP increased significantly (Table 2). Similar to our results, decrease in yield and yield attributing traits in rice varieties and mutants in response to salt stress were also reported by others (40, 42, 43). Importantly, the highest reduction was found in sensitive variety and mutants (BRRI dhan28, Line-18 and Line-65) and the lowest reduction was found in tolerant genotype Binadhan-10 and in the mutant Line-1 (Table 2). Similarly, an increase in NUFP in response to salt stress was also observed by Rahman *et al.* (33). The phenological appearance of the panicles of the rice plants grown under control and salt stress also corroborate with the yield contributing traits (Fig. 4). Limited nutrition and carbohydrate translocation to the panicles, pollen viability and stigma receptivity disruption, and excessive Na^+ and Cl^- buildup in floral sections could all contribute to the increase of NUFP or decrease yield (44). Early leaf senescence caused by salt stress in rice plants increased Na^+ accretion, which decreased panicle development and assimilated production, lowering growth and yield attributes. In addition to lowering photosynthetic pigment levels, salt stress also affects osmolytes accumulation, plant water interactions, membrane integrity, and K^+ uptake, which lowers yield and yield characteristics (45).

Fig 4. Phenotypic appearances of mature panicles after imposition of salt stress at the reproductive stage. [Here, 28= BRRI dhan28, 10= Binadhan-10, 1= Line-1, 18= Line-18, 65= Line-65].

Estimation of Correlation Co-efficient

Grain yield is a complex character resulting from multiplicative interactions among different yield attributing traits. In selection breeding program, the selection efficiency depends on the knowledge of relationship among traits. In the current study, GYP under control condition showed a significant positive correlation with PL, NFGP, NUGP, SF% and 100-SW whereas under saline environment, GYP showed a significant positive correlation with PL, NFGP, and 100-SW (Table 3). Similar positive correlation of GYP with PL, NFGP, NUGP, SF% and 100-SW was also reported by others under control conditions (42). Therefore, these traits could be utilized in indirect selection so as to improve yield. We observed a little difference among the relation of the traits under control and salt stress conditions. These results indicate that indirect selection strategy for rice genotypes under salt stress or non-stress environment should be different (43).

Principal Component Analysis

Principal component analysis helps to categorize the genotypes based on their responses towards the studied variables. Previous researchers also used principal component analysis to explore the salinity tolerance of corn (*Zea mays* L.), mungbean (*Vigna radiata* (L.) Wilczek), wheat (*Triticum aestivum* L.) (46–48). In this study, the first two principal components accounted for 74.9% of the total variation in the entire dataset (Table 4; Fig. 2). Here, PC1 and PC2 revealed that, SFW, SF%, RL, SPAD, SL, SDW, RDW, 100-SW, RL, YPP, PL, NFGP and NUGP were the most important traits responsible for the variation (Table 4). This suggests that the research accessions were much diversified in terms of the majority of the quantitative traits that were assessed. Importance of SFW, SDW, SL, chlorophyll content, and RDW, RFW in explaining variation in salinity tolerance was also reported by Tabassum *et al.* (49). Rahman *et al.* (33) also found negative correlation of PC1 with NUGP and positive correlation with YPP, SF%, NFGP, DM and 100-SW.

Interestingly, the PC1 is clearly separated the stressed samples from the unstressed ones in our study (Fig. 2). Among the unstressed samples, the tolerant check Binadhan-10 and the susceptible check BRRI dhan28 were placed furthest apart in the same quadrant of the biplot (Fig. 2). Similar positioning was also observed for the stressed samples of these two contrastingly tolerant checks in the opposite quadrant. Among the lines that were tested for salinity tolerance, the Line-1 which showed reasonable degree of salt tolerance was placed in-line with the resistant check Binadhan-10 for both stressed and unstressed samples in the respective quadrant of the biplot (Fig. 2). The positioning of the susceptible check BRRI dhan28 in further apart from that of tolerant lines can be attributed to the higher reduction in SFW (the parameter having highest component loading), SDW and YPP upon stress imposition (Table 1 & 2).

Stress Tolerance Indices in Rice Genotypes Estimated from Grain Yield per Panicle

According to Rosielle and Hamblin (17) and Bouslama and Schapaugh (19), the genotypes having higher values of MP, GMP, STI and YSI are considered as the more resistant genotypes. However according to Krishnamurthy *et al.* (50), higher TOL and SSI values suggest relative greater sensitivity to stress; conversely, a lower TOL and SSI value for a particular genotype denotes greater genetic stability in both stressful and non-stressful conditions. Genotypes with high yields under stressful circumstances are favoured via selection based on these two criteria. According to a study, there is a positive association between MP and YS (a stressful environment), hence choosing plants based on MP will increase average yields in both stressful and non-stressful environments. According to the GMP investigation, genotype Line-65 had the greatest value (Table 5). According to the SSI investigation, the genotypes Line-65 and Binadhan-10 had the highest values and Line-1 and Line-65 had the lowest values (Table 5). Higher SSI readings suggest a greater sensitivity to and yield decline when exposed to salt stress. Binadhan-10 and Line-1 could be regarded as tolerant genotypes as a result of SSI. The genotype Line-65 had the highest TOL value, while the genotype Binadhan-10 had the lowest value, according to the TOL index results. Higher tolerance to salt stress is indicated by a low TOL index value. In particular, genotypes with low yield potential in non-stressful situations and superior yield potential under stressful ones are selected on the basis of this criterion (16). Therefore, this criterion does not assist us in differentiating between genotypes that yield well under stress and genotypes that yield well under both stress and unstressed conditions. In a challenging environment, a genotype with a higher STI value will be more resilient to stress and have higher yield potential. The highest STI value was found in Line-65 followed by Line-1, Binadhan-10, Line-18 and BRRI dhan28 (Table 5). In the present study, the highest YSI was observed in Binadhan-10 followed by Line-1, Line-18, BRRI dhan28 and Line-65. So, Binadhan-10 and Line-1 could be considered as salt tolerant according to this indicator. YSI was particularly relevant to differentiate tolerant and sensitive varieties under saline conditions. Based upon the stress tolerance indices it was found that Line-65 had the highest values of MP, GMP, SSI, STI and TOL and the lowest values of YSI (Table 5). The highest values of MP, GMP and STI indicate tolerance to salt and high yield potential. But the highest values of SSI, TOL and lowest values of YSI indicates the sensitiveness of Line-65 to salt stress. So, it can be concluded that Line-65 had greater high yield potential but highly susceptible to salt stress. On the other hand, Line-1 and Binadhan-10 showed higher values of MP, GMP, STI and YSI and lower values of TOL and SSI which indicate tolerance to salt stress of these genotypes (Table 5). Therefore, among three advanced mutant lines, Line-1 showed better salt tolerance capability at the reproductive stage according to stress tolerance indices. Using STI, several other studies were also successful in identifying salt-tolerant genotypes in wheat (*Triticum aestivum*), rice (*Oryza sativa*), barley (*Hordeum vulgare*), and watermelon (*Citrullus lanatus*) (22).

Conclusion

The imposition of salinity stress at the seedling and reproductive stage led to a significant decrease in seedling growth and yield attributing traits for most of the mutants whereas the NUGP and Na^+/K^+ ratio was increased. Importantly, salinity tolerant Binadhan-10 and mutant Line-1 showed limited increase or decrease of the traits which is also evident from the biplot of PCA. Phenotypic correlation studies at the reproductive stage revealed that GYP showed significant positive correlation with PL, NFGP and 100-SW under saline stress conditions. Thus, attention should be given to those traits when selecting the salt tolerant genotypes. The stress tolerance indices *viz*., SSI and TOL showed higher values for sensitive genotypes while YSI, STI, MP and GMP showed higher values for tolerant genotypes. According to stress tolerance indices, Line-65 was categorized as high yielding while Binadhan-10 and Line-1 were categorized as reproductive-stage saline tolerant genotypes. So, based on the research findings of seedling and reproductive stage phenotyping, Line-1 can be considered as a salt tolerant advanced mutant line. However, further studies should be conducted with Line-1 for isolating specific gene(s) or QTLs conferring salt tolerance at the various phases of growth as well as utilizing this genotype as a probable candidate of donor parents to develop salt-tolerant high-yielding rice varieties.

Acknowledgements

The authors gratefully acknowledge BRRI and BINA personnel for providing seeds. The authors also acknowledge the financial support provided by the Ministry of Education (MOE), Government of the People's Republic of Bangladesh (Grant No. 2018/518/MOE), Ministry of Science and Technology (Grant No. 221132), and BAURES, BAU (Grant No. 2022/72/BAU) to conduct this project work.

Authors' contributions

Conceptualization and methodology: TK and MAH; Experimentation and formal analysis: TK, SMK, MHM, JN, and SAJ; Data analysis: TK and SAJ; Original draft preparation: TK, SMK, MHM and SAJ; Edited Table and Figure: TK and MAE; Writing, review and editing:, MRH, MAE and MAH; Supervision: MRH and MAH; Project administration: MAH. All authors have read and approve the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

References

1. Jaiswal S, Gautam R, Singh R, Krishnamurthy S, Ali S, Sakthivel K, Iquebal M, Rai A, Kumar D. Harmonizing technological advances in phenomics and genomics for enhanced salt tolerance in rice from a practical perspective. Rice. 2019;12:1-19. https://doi.org/10.1186/s12284-019-0347-1.
2. Ganie SA, Molla KA, Henry RJ, Bhat KV, Mondal TK. Advance in understanding salt tolerance in rice. Theoretical and Applied Genetics. 2019;132(4):851-70. https://doi: 10.1007/s00122-019-03301-8.
3. Food and Agriculture Organization (FAO). Production/Yield Quantities of Rice, Paddy in World. Available online: http://www.fao.org/faostat/en/#data/QC/visualize (accessed on 3 August 2020). Food and Agriculture Organization of the United Nations. 2020.
4. Salehin M, Chowdhury MMA, Clarke D, Mondal S, Nowreen S, Jahiruddin M, Haque A. Mechanisms and drivers of soil salinity in Coastal Bangladesh. In: Nicholls R, Hutton CW, Adger W, Hanson S, Rahman M, Salehin M, editors. Ecosystem services for well-being in deltas. Cham: Palgrave Macmillan. 2018;p. 333-47. https://doi.org/10.1007/978-3-319-71093-8_18
5. Dasgupta S, Hossain MM, Huq M, Wheeler D. Climate change, salinization and high-yield rice production in coastal Bangladesh. Agricultural and Resource Economics Review. 2018;47(1):66-89. https://doi.org/10.1017/age.2017.14.
6. Rao PS, Mishra B, Gupta SR, Rathore A. Reproductive stage tolerance to salinity and alkalinity stresses in rice genotypes. Plant Breeding. 2008;127(3):256-61.https://doi.org/10.1111/j.1439-0523.2007.01455.x
7. Mostofa MG, Hossain MA, Fujita M. Trehalose pretreatment induces salt tolerance in rice seedlings: Oxidative damage and co-induction of antioxidant defense and glyoxalase systems. Protoplsasma. 2015;252(2):461-75. https://doi.org/10.1007/s00709-014-0691-3.
8. Kamran M, Parveen A, Ahmar S, Malik Z, Hussain S, Chattha MS. An overview of hazardous impacts of soil salinity in crops, tolerance mechanisms and amelioration through selenium supplementation. International Journal of Molecular Sciences. 2020;21:148. https://doi.org/10.3390/ijms21010148.
9. Acosta-Motos JR, Ortuño MF, Bernal-Vicente A, Diaz-Vivancos P, Sanchez-Blanco MJ, Antonio-Hernandez J. Plant responses to salt stress: adaptive mechanisms. Agronomy. 2017;7:18. https://doi.org/10.3390/agronomy7010018
10. Assaha DVM, Ueda A, Saneoka H, Yahyai RA, Yaish MW. The role of Na^+ and K^+ transporters in salt stress adaptation in glycophytes. Frontiers in Physiology. 2017;8:509. https://doi.org/10.3389/fphys.2017.00509.

11. Ashraf M, Foolad MR. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany. 2007;59:206-16. http://dx.doi.org/10.1016/j.envexpbot.2005.12.006.
12. Singh RK, Kota S, Flowers TJ. Salt tolerance in rice: Seedling and reproductive stage QTL mapping come of age. Theoretical and Applied Genetics. 2021;134:3495-33. https://doi.org/10.1007/s00122-021-03890-3.
13. Joshi R, Prasat R, Sharma PC, Singla-pareek SL, Pareek A. Physiological characterization of gamma ray induced mutant population of rice to facilitate biomass and yield improvement under salinity stress. Indian Journal of Plant Physiology. 2016;21(4):545-55. https://doi.org/10.1007/s40502-016-0264-x.
14. Chowdhury N, Islam S, Mim MH, Akter S, Naim J, Nowicka B, Hossain MA. Characterization and genetic analysis of the selected rice mutant populations. SABRAO Journal of Breeding and Genetics. 2023;55(1):25-37. http://doi.org/10.54910/sabrao2023.55.1.
15. Thomas RL, Sheard RW, Moyer JR. Comparison of conventional and automated procedures for nitrogen, phosphorous and potassium analysis of plant material using a single digestion. Agronomy Journal. 1967;59(3):240-43. https://doi.org/10.2134/agronj1967.00021962005900030010x.
16. Fernandez GC. Effective selection criteria for assessing plant stress tolerance. In: Kuo CG, editor. Proceedings of the international symposium on adaptation of vegetables and other food crops in temperature and water stress. Tainan: AVRDC Publication. 1992; p. 257-70.
17. Rosielle AA, Hamblin J. Theoretical aspects of selection for yield in stress and non-stress environments. Crop Science. 1981;21:943-46. http://dx.doi.org/10.2135/cropsci1981.0011183X002100060033x.
18. Fischer KS, Maurer R. Drought resistance in spring wheat cultivars. I. Grain yield responses. Australian Journal of Agricultural Research. 1978;29:897-912. http://dx.doi.org/10.1071/AR9780897.
19. Bouslama M, Schapaugh WT. Stress tolerance in soybeans: Evaluation of three screening techniques for heat and drought tolerance. Crop Science. 1984;24(5):933-37. https://doi.org/10.2135/cropsci1984.0011183X002400050026x.
20. Haque MA, Rafii MY, Yusoff MM, Ali NS, Yusuff O, Datta DR, Anisuzzaman M, Ikbal MF. Advanced breeding strategies and future perspectives of salinity tolerance in rice. Agronomy. 2021;11(8):1631. https://doi.org/10.3390/agronomy11081631
21. Kakar N, Jumaa SH, Redoña ED, Warburton ML, Reddy KR. Evaluating rice for salinity using pot-culture provides a systematic tolerance assessment at the seedling stage. Rice. 2018;12:57. https://doi.org/10.1186/s12284-019-0317-7.
22. Rasel M, Tahjib-Ul-Arif M, Hossain MA, Sayed MA, Hassan L. Discerning of rice landraces (*Oryza sativa* L.) for morpho-physiological, antioxidant enzyme activity and molecular markers responses to induced salt stress at the seedling stage. Journal of Plant Growth Regulation. 2021;39:41-59. https://doi.org/10.1007/s11738-018-2645-4.
23. Rahnama A, James AR, Poustini K, Munns R. Stomatal conductance as a screen for osmotic stress tolerance in durum wheat growing in saline soil. Functional Plant Biology. 2010;37(3):255-63. https://doi.org/10.1071/FP09148.
24. Munns R, Tester M. Mechanisms of salinity tolerance. Annual Review of Plant Biology. 2008;59:651-81. https://doi.org/10.1146/annurev.arplant.59.032607.092911.
25. Castillo EG, Tuong TP, Ismail AM, Inubushi K. Response to salinity in rice: Comparative effects of osmotic and ionic stresses. Plant Production Science. 2007;10:159-70. https://doi.org/10.1626/pps.10.159.
26. Zhang Y, Fang J, Wu X, Dong L. Na^+/K^+ balance and transport regulatory mechanisms in weedy and cultivated rice (*Oryza sativa* L.) under salt stress. BMC Plant Biology. 2018;18:1-14. https://doi.org/10.1186/s12870-018-1586-9.
27. Omisun T, Sahoo S, Saha B, Panda SK. Relative salinity tolerance of rice cultivars native to North East India: A physiological, biochemical and molecular perspective. Protoplasma. 2018;255:193-202. https://doi.org/10.1007/s00709-017-1142-8.
28. Mahmood U, Hussain S, Hussain S, Ali B, Ashraf U, Zamir S. Morpho-physio-biochemical and molecular responses of maize hybrids to salinity and waterlogging during stress and recovery phase. Plants. 2021;10:1345. https://doi.org/10.3390/plants10071345.
29. Nahar L, Aycan M, Hanamata S, Baslam M, Mitsui T. Impact of single and combined salinity and high-temperature stresses on agrophysiological, biochemical and transcriptional responses in rice and stress release. Plants. 2022;11:501. https://doi.org/10.3390/plants11040501.
30. Rasel M, Hassan L, Hoque MIU, Saha SR. Estimation of genetic variability, correlation and path coefficient analysis in local landraces of rice (*Oryza sativa* L.) for the improvement of salinity tolerance. Journal of Bangladesh Agricultural University. 2018;16(1):41-46. doi: 10.3329/jbau.v16i1.36479
31. Faseela P, Sinisha AK, Brestic M, Puthur JT. Chlorophyll a fluorescence parameters as indicators of a particular abiotic stress in rice. Photosynthetica. 2019;57:108-15. https://doi.org/10.32615/ps.2019.147.
32. Moradi F, Ismail AM. Responses of photosynthesis, chlorophyll fluorescence and ROS-scavenging systems to salt stress during seedling and reproductive stages in rice. Annals of Botany. 2007;99(6):1161-73. https://doi.org/10.1093/aob/mcm052.
33. Rahman MM, Jahan I, Al Noor MM, Tuzzohora MF, Sohag AAM, Raffi SA, Islam MM, Burritt DJ, Hossain MA. Potential determinants of salinity tolerance in rice (*Oryza sativa* L.) and modulation of tolerance by exogenous ascorbic acid application. Journal of Phytology. 2020;12:86-98. https://doi.org/10.25081/jp.2020.v12.6535.
34. Munns R, James RA, Läuchli A. Approaches to increasing the salt tolerance of wheat and other cereals. Journal of Experimental Botany. 2006;57:1025-43. https://doi.org/10.1093/jxb/erj100.
35. Flexas J, Diaz-Espejo AN, Galmes J, Kaldenhoff R, Medrano H, Ribas-Carbo MI. Rapid variations of mesophyll conductance in response to changes in CO_2 concentration around leaves. Plant, Cell and Environment. 2007;30:1284-98. https://doi.org/10.1111/j.1365-3040.2007.01700.x.
36. Munns R, James RA, Gilliham M, Flowers TJ, Colmer TD. Tissue tolerance: An essential but elusive trait for salt-tolerant crops. Functional Plant Biology. 2016;43:1103-13. https://doi.org/10.1071/FP16187
37. Roy SJ, Negrão S, Tester M. Salt resistant crop plants. Current Opinion in Biotechnology. 2014;26:115-24. https://doi.org/10.1016/j.copbio.2013.12.004.
38. Mitsuya S, Yano K, Kawasaki M, Taniguchi M, Miyak H. Relationship between the distribution of Na^+ and the damages caused by salinity in the leaves of rice seedlings grown under a saline condition. Plant Production Science. 2002;5(4):269-74. https://doi.org/10.1626/pps.5.269.
39. Ismail AM, Horie T. Genomics, physiology and molecular breeding approaches for improving salt tolerance. Annual Review of Plant Biology. 2017;68(1):405-34. https://doi.org/10.1146/annurev-arplant-042916-040936.
40. Rao PS, Mishra B, Gupta SR, Rathore A. Reproductive stage tolerance to salinity and alkalinity stresses in rice genotypes. Plant Breeding. 2008;127(3):256-61. https://doi.org/10.1111/j.1439-0523.2007.01455.x.

41. Chattopadhyay K, Nayak AK, Marndi BC, Poonam A, Chakraborty K, Sarkar RK. Novel screening protocol for precise phenotyping of salt-tolerance at reproductive stage in rice. Physiology and Molecular Biology of Plants. 2018;24(6):1047-58. https://doi.org/10.1007/s12298-018-0591-7.

42. Gerona MEB, Deocampo MP, Egdane JA, Ismail AM, Dionisio-Sese ML. Physiological responses of contrasting rice genotypes to salt stress at reproductive stage. Rice Science. 2019;26(4):207-19. https://doi.org/10.1016/j.rsci.2019.05.001

43. Krishnamurthy SL, Gautam RK, Sharma PC, Sharma DK. Effect of different salt stresses on agro-morphological traits and utilisation of salt stress indices for reproductive stage salt tolerance in rice. Field Crop Research. 2016;190:26-33. https://doi.org/10.1016/j.fcr.2016.02.018.

44. Abdullah Z, Khan MA, Flowers TJ. Causes of sterility in seed set of rice under salinity stress. Journal of Agronomy and Crop Science. 2001;187(1):25-32. https://doi.org/10.1046/j.1439-037X.2001.00500.x.

45. Al-Ashkar I, Alderfasi A, El-Hendawy S, Al-Suhaibani N, El-Kafafi S, Seleiman MF. Detecting salt tolerance in doubled haploid wheat lines. Agronomy. 2019;9:211. https://doi.org/10.3390/agronomy9040211.

46. Jahan I, Rahman MM, Tuzzohora1 MF, Hossain MA, Begum SN, Burritt DJ, Hossain MA. Phenotyping of mungbean (*Vigna radiata* L.) genotypes against salt stress and assessment of variability for yield and yield attributing traits. Journal of Plant Stress Physiology. 2020;6:07-17. https://doi.org/10.25081/jpsp.2020.v6.6111.

47. Robin AHK, Matthew C, Uddin MJ, Bayazid KN. Salinity-induced reduction in root surface area and changes in major root and shoot traits at the phytomer level in wheat. Journal of Experimental Botany. 2016;67(12):3719-29. https://doi.org/10.1093/jxb/erw064.

48. Wijewardana C, Henry WB, Hock MW, Reddy KR. Growth and physiological trait variation among corn hybrids for cold tolerance. Canadian Journal of Plant Science. 2016;96:639-56.http://doi.org/10.1139/CJPS-2015-0286.

49. Tabassum R, Tahjib-Ul-Arif M, Hasanuzzaman M, Sohag AAM, Islam MS, Shafi SMSH, Islam MM, Hassan L. Screening salt-tolerant rice at the seedling and reproductive stages: An effective and reliable approach. Environmental and Experimental Botany. 2021;192:104629. https://doi.org/10.1016/j.envexpbot.2021.104629.

50. Krishnamurthy SL, Sharma SK, Gautam RK, Kumar V. Path and association analysis and stress indices for salinity tolerance traits in promising rice (*Oryza sativa* L.) genotypes. Cereal Research Communication. 2014;42(3):474-83. https://doi.org/10.1556/CRC.2013.0067.